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<b>(21) International Application Number:</b> PCT/NL93/00025 <b>(22) International Filing Date:</b> 25 January 1993 (25.01.93)  <b>(30) Priority data:</b> 92200210.0 24 January 1992 (24.01.92) EP <b>(34) Countries for which the regional or international application was filed:</b> NL et al.  <b>(71) Applicant (for all designated States except US):</b> GIST-BROCADES N.V [NL/NL]; Wateringseweg 1, P.O. Box 1, NL-2600 MA Delft (NL).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> HAMSTRA, Reinder, Sietze [NL/NL]; Karperdaal 167, NL-2553 PE Den Haag (NL). TROMP, Augustinus, Franciscus, Cornelis, Petrus, Maria [NL/NL]; Aert van Neslaan, NL-2341 HX Oegstgeest (NL).		<b>(74) Agents:</b> MATULEWICZ, Emil, Rudolf, Antonius et al.; Gist-Brocades N.V., Patents and Trademarks Department, Wateringseweg 1, P.O. Box 1, NL-2600 MA Delft (NL).  <b>(81) Designated States:</b> CA, JP, NO, US.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> METHOD FOR THE PREPARATION OF FEED PELLETS  <b>(57) Abstract</b>  The present invention discloses a method for obtaining feed pellets. The method comprises the addition of active ingredients to feed pellets after the pellets have been extruded. The method further comprises the addition of a solution or suspension of the desired feed or ingredient in a water or oil phase to the pellets under reduced pressure and subsequently increasing the pressure. The method results in pellets with a high degree of loading and in which the active ingredients are homogeneously distributed.		

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Method for the preparation of feed pellets

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Field of the invention

The present invention relates to a method for preparing feed pellets. The process for the addition of sensitive ingredients during the preparation of pellets is so altered that activity losses are minimized. Specifically, the pellets are treated by mixing a suspension or solution of active ingredients in a fluid phase (for example oil or water) under appropriate conditions with carrier material which has been previously granulated.

Background of the invention

Feed is generally prepared by mixing different ingredients which are found to be necessary (active ingredients) with carrier materials essential to obtain the feed in the desired form. The desired form may be a powder, a pellet, a solution or a suspension. The preferred form will depend on the application conditions, the composition and the transport.

A well known problem in feed preparation is the loss of active substance during the pelleting process, especially when extrusion is used. Extensive research results have been published describing the loss of activity of the active ingredients during extrusion cooking.

Lee et al. (AIChE Symposium Series (1978) 172 : 192-195) investigated the stability of vitamin A in extrusion cooking processing. The percent of retention of the different tested forms of vitamin A ranged from 50 to 100%. This was reported to be relatively stable when compared with the retention of cantaxanthin which was reported to be in the range of 30 to 35%. Berset (Ind. Aliment. Agric. (1987) 104 : 529-533) reports a loss of 18% of cantaxanthin when a

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commercial preparation was treated. Hencken and Estermann (Aquaculture Ind. Develop. Report (1991) 91 : 34-51) report a 25 to 48% loss of activity of synthetic astaxanthin during fish feed preparation using extrusion cooking.

5 A possible solution to this loss of active substance during granulation is to add the active ingredient in a suitable form like a suspension or solution in a fluid phase subsequent to the granulation step. Mixing of components with a fluid phase can be difficult since active ingredients are  
10 not always soluble. Furthermore, the addition after the extrusion step makes it more difficult to obtain a homogenous product.

High concentrations of active ingredients in feed for cattle, poultry or fish in a form which is easy to handle and  
15 easy to quantify can be obtained by soaking granulated particles with a suspension or solution containing the desired feed or ingredients thereof. The granulated material contains pores which are filled with the solution or suspension and the loaded pellets can easily be used.

20 The loading of the pellets is generally performed at atmospheric pressure. The process poses some severe drawbacks:

- it is difficult to reproduce the amount of suspension or solution which is absorbed into the pellets, this is  
25 especially relevant if the aim is to control the amount of fluid (oil etc.) which is fed to the animal concerned,
- the pellets are generally preferentially covered at their surface, which may cause problems (like oxidation) of the active ingredient,
- 30 - the particles in the suspension may block the pores leading to an inefficient use of the particle pore volume and to the above mentioned surface phenomena.

There is thus a lack of reproducibility in dosage of the active substance and a higher chance to losses of active  
35 ingredient as a consequence of insufficient penetration of the active ingredient in the feed granule.

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To avoid these problems granulated material with wide pores can be used but this evidently leads to loss of strength of the loaded pellets.

The present invention overcomes the problems described  
5 above.

#### Summary of the invention

10 The present invention discloses a method for minimizing the loss of activity of sensitive materials due to the well known extrusion process. The invention discloses that this can be done by addition of the material after the extrusion process. The invention is exemplified by the addition of  
15 astaxanthin in oil to feed pellets. The invention thus discloses a method for preparing feed pellets loaded with an active ingredient comprising the addition of active material after the extrusion process. The active ingredient is an enzyme, a vitamin, a pigment or a carotenoid. Preferably, the  
20 active ingredient is astaxanthin.

The present invention further provides a method for preparing pellets which are homogenously loaded with active ingredient. The method comprises the addition of a suspension or solution of the active ingredients to pellets under  
25 reduced pressure and subsequently increasing the pressure. Preferably, the addition is performed under vacuum.

The present invention also provides feed pellets which have been obtained by this method. Specifically, fish meal pellets soaked with an oil suspension containing astaxanthin  
30 are disclosed. Such pellets can be recognized by their high degree of loading which is moreover relatively homogenous. A fish pellet is disclosed which contains more than 81.5 mg astaxanthin per kg dry matter.

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Detailed description of the Figures

Figure 1 shows the spraydried Phaffia rhodozyma suspended in oil after one (A), two (B) and three (C) passes through a bead mill.

Figure 2 shows the results of the addition of the suspensions of Figure 1 (A), (B) and (C) under atmospheric pressure to the fish meal pellets.

Figure 3 shows the results of the addition of the suspensions of Figure 1 (A), (B) and (C) under vacuum to the fish meal pellets.

Detailed description of the invention

The present invention discloses a method for preparing feed pellets. The present invention discloses how the loss of activity of sensitive materials due to the well known extrusion process can be minimized. The invention discloses a method for preparing feed pellets loaded with an active ingredient comprising the addition of active material after the extrusion process. The extrusion process can also be the so-called extrusion cooking process. The active ingredient is an enzyme or protein, a pigment or a carotenoid.

The addition of the active ingredient (or any ingredient which can be damaged by the extrusion process) after the extrusion process may cause a problem due to the fact that the extrusion process also serves to obtain a homogenous feed pellet. This problem can be circumvented by the addition of a suspension or solution of feed or ingredients thereof in a fluid phase to the pellets under reduced pressure and subsequently increasing the pressure. The reduced pressure can be vacuum.

The fluid phase can be any fluid (for example water or oil) provided that the active ingredient can be dispersed or dissolved in this fluid. In the present examples capelin-oil is used.

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The present invention discloses a method for preparing pellets loaded in a controlled manner with active ingredients. The method comprises the addition under mixing of a suspension or solution of feed or ingredients thereof in  
5 a fluid phase to the pellets under vacuum and subsequently releasing the vacuum.

The pellets are composed of an edible material. The specific composition depends on the desired characteristics of the material. The choice of the pellet, volume, weight and  
10 pore diameter also depends on the kind of feed one wants to use and on the application for which the loaded pellets are meant. A preferred pellet material in the present invention is fish meal.

The feed or feed ingredient may be any ingredient that  
15 is needed. The choice may depend on the nutritional value or on certain rheological characteristics which may be obtained by the activity of the ingredient. In the following some examples of feed or feed ingredients will be discussed. In general terms enzymes or proteins, pigments, vitamins,  
20 antioxidants, colouring agents and carotenoids can be employed. Obviously combinations of these ingredients can be added, simultaneously or successively.

#### a) Enzymes

25 In general all feed enzymes can be used in the present method these include phytase, amylase and protease. Amylase may be brought on the pellet and subsequently the pellets can be mixed with feed. It is also possible to add the enzyme suspension after pelletizing/extrusion. The activity of this  
30 enzyme can be quantified and the added amount controlled. The enzyme can change the fluidity of the feed to which it is added or improve the digestability of feed components. Another example of an enzyme is phytase here again the dosage can be controlled by the method of the present invention.



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## b) Carotenoids

Astaxanthin is used as a natural colourant for salmonids. Astaxanthin cannot be solubilized in a water phase. A suspension of astaxanthin in oil or a suspension of Phaffia rhodozyma cells (or cell fragments) containing astaxanthin in oil, can be used to soak the pellets. Incidentally, it was found that this astaxanthin contrary to the commercial synthetic astaxanthin as used by Hencken and Estermann (1991, opt. cit.) could form a stable suspension in oil.

The amount of oil and astaxanthin fed to the fish can thus be controlled. The control of the amount of oil provides a way of influencing the growth rate of the fish. Other carotenoids which can advantageously be used in the method of the present invention include  $\beta$ -carotene, cantaxanthin and zeaxanthin.

Desired pellets are brought under vacuum and the liquid phase which may be a solution or suspension of desired feed or ingredients thereof or other ingredients in a water or oily phase is added. The equipment used can range from a waterjet and a flask which can be vacuated at laboratory scale via a Rotorvapor to large scale equipment.

The pellets obtained by the present method show a high degree of loading, moreover the pellets are loaded relatively homogenously. Furthermore, the amount of loading can be regulated by regulating the amount of fluid phase added. This is especially important if one wants to regulate the amount of oil provided to the desired animal eg. fish.

The present invention discloses for example fish meal pellets which contain more than 30 mg preferably more than 81.5 mg astaxanthin per kg of dry matter.

The present invention thus provides a method for preparing feed pellets wherein the active ingredient is added after the extrusion. The feed pellets are subsequently loaded



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under reduced pressure which makes possible an accurate,  
reproducible and homogenous loading.

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Example IPreparation of an oil suspension containing astaxanthin

5        A Phaffia rhodozyma culture was centrifuged and dried  
by spraydrying. The spraydried powder ( $d_p = 100 \mu m$ ) was  
suspended in a commercial fish-oil (Capelin oil). The mixture  
of cell material containing astaxanthin and oil was ground in  
a bead mill. After one pass of milling the particle diameter  
10 was determined. Microscopic analysis indicated that a large  
proportion of the spraydried powder particles were  
disintegrated. A small portion of the particles (<5%) was  
unaffected and had a diameter of about  $100 \mu m$  (Fig. 1A). The  
astaxanthin concentration was about 630 ppm (Suspension I).

15        A second suspension was prepared (Suspension II) by  
grinding the particles three times in a bead mill (Fig. 1C).  
Microscopic analysis revealed that practically all cells were  
disintegrated, the largest agglomerates of cells had a  
diameter of less than  $20 \mu m$ .  
20 The astaxanthin concentration was 330 ppm.

Example IISoaking of fish meal pellets

25        Commercial fish meal pellets were obtained from Trouw  
International (Putten, the Netherlands). These pellets were  
semi-manufactured, which means that they had not been treated  
30 with oil. These pellets have a diameter of 8mm, a length of  
between 0.8 and 1.2 mm and a weight of between 220 and 330  
mg.

Further experiments have all been performed in duplo, data  
reported are the mean values.

35

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## A. Soaking at atmospheric pressure

100 g of fish meal pellets were brought into a 1 L beaker. 25 g of an oil suspension containing about 8 mg astaxanthin, was added (Suspension II or twice diluted  
5 Suspension I). Mixing was performed during 1 hour in a Turbula mixer.

## B. Soaking under vacuum

100 g fish meal pellets were dried under vacuum for 30  
10 min. at 60°C in a 500ml flask in a Rotorvapor. Vacuum was obtained using a waterjet. 25 g of an oil suspension containing astaxanthin was added (as above) in small amounts with vacuum application in between. Rotation was continued for 10 min. and the flask was subsequently cooled on an ice  
15 bath.

Example IIIAnalysis of soaked pellets

20

## A. Microscopic analysis

When pellets were prepared at atmospheric pressure the pellets obtained using Suspension I mainly gave a red colour (astaxanthin) at the surface (Figure 2 A). Pellets obtained  
25 using Suspension II showed a red colour also in the interior but the concentration at the surface was higher (Figure 2 B and C).

When pellets were prepared under vacuum Suspension I  
30 gave pellets with red colour in the interior and Phaffia mainly at their surface (Figure 3 A). With Suspension II the red colour was mainly in the interior of the pellet (Figure 3 B and C).

35 Reduction of particle size and application of the Suspension under vacuum clearly gives a better reproducible result.

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## B. Washing test

The release of astaxanthin from the particles was simulated by washing the pellets. The amount of astaxanthin was determined using HPLC.

5 Experiments were performed as follows.

- a) 50 g fish meal pellet was brought in a 250 ml flask,
- b) 100 ml water of 10°C was added,
- c) the flask was put in a Shake Water Bath at 10°C, 75 strokes per min. 4.5 cm amplitude for 5 min.
- 10 d) after shaking the particles were immediately sieved over a sieve with holes of 1mm diameter,
- e) the astaxanthin concentration was determined in untreated pellets, washing water and in the washed pellets, using HPLC.

15

The following amounts of astaxanthin were found.

## A. Atmospheric pressure

20 Table I

	Suspension I	dry matter	asta mg/kg	asta mg/kg dry matter	asta loss
25	Pellets	0.94	73.0	77.6	
	Washwater		1.3	1.5	1.9 %
	Washed pellets	0.83	66.0	79.5	

30

Table II

	Suspension II	dry matter	asta mg/kg	asta mg/kg dry matter	asta loss
35	Pellets	0.95	74.8	78.7	
	Washwater		0.28	0.4	0.5 %
	Washed pellets	0.82	66.9	81.5	

40

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## B. Under vacuum

5

Table III

	Suspension I	dry matter	asta mg/kg	asta mg/kg dry matter	asta loss
10	Pellets	0.94	82.0	86.3	
	Washwater		1.5	1.6	1.8 %
	Washed pellets	0.86	73.0	84.8	

15

Table IV

	Suspension II	dry matter	asta mg/kg	asta mg/kg dry matter	asta loss
20	Pellets	0.95	82.4	86.7	
	Washwater		0.16	0.2	0.2 %
	Washed pellets	0.75	64.2	85.6	

25

It is clear that the loss of astaxanthin is lower when particle diameter is smaller (Suspension II). And that the loss is further reduced considerably when pellets are  
30 prepared under vacuum.

The above examples merely serve to illustrate the principle underlying the invention and in no way are meant to determine the scope of the disclosure.

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Claims

1. A method for preparing feed pellets loaded with an active ingredient comprising the addition of active material  
5 after the extrusion process.

2. A method according to claim 1 wherein the active ingredient is an enzyme or protein, a pigment or a carotenoid.

10

3. A method for preparing pellets loaded with an active ingredient comprising the addition of a suspension or solution of feed or ingredients thereof in a fluid phase to the pellets under reduce pressure and subsequently increasing  
15 the pressure..

4. A method according to claim 3 wherein the reduced pressure is vacuum.

20

5. A method according to claim 3 or 4 characterized in that the fluid phase is water or oil.

6. A method according to claim 3 to 5 characterized in that the active ingredient is an enzyme, a pigment or a  
25 carotenoid.

7. A method according to claim 6 characterized in that the enzyme is selected from the group comprising phytase, lactase, protease and amylase.

30

8. A method according to claim 6 characterized in that the carotenoid is selected from the group comprising astaxanthin,  $\beta$ -carotene, cantaxanthin and zeaxanthin.

35

9. A feed pellet characterized in that it contains a high amount of astaxanthin which is homogenously distributed throughout the pellet.

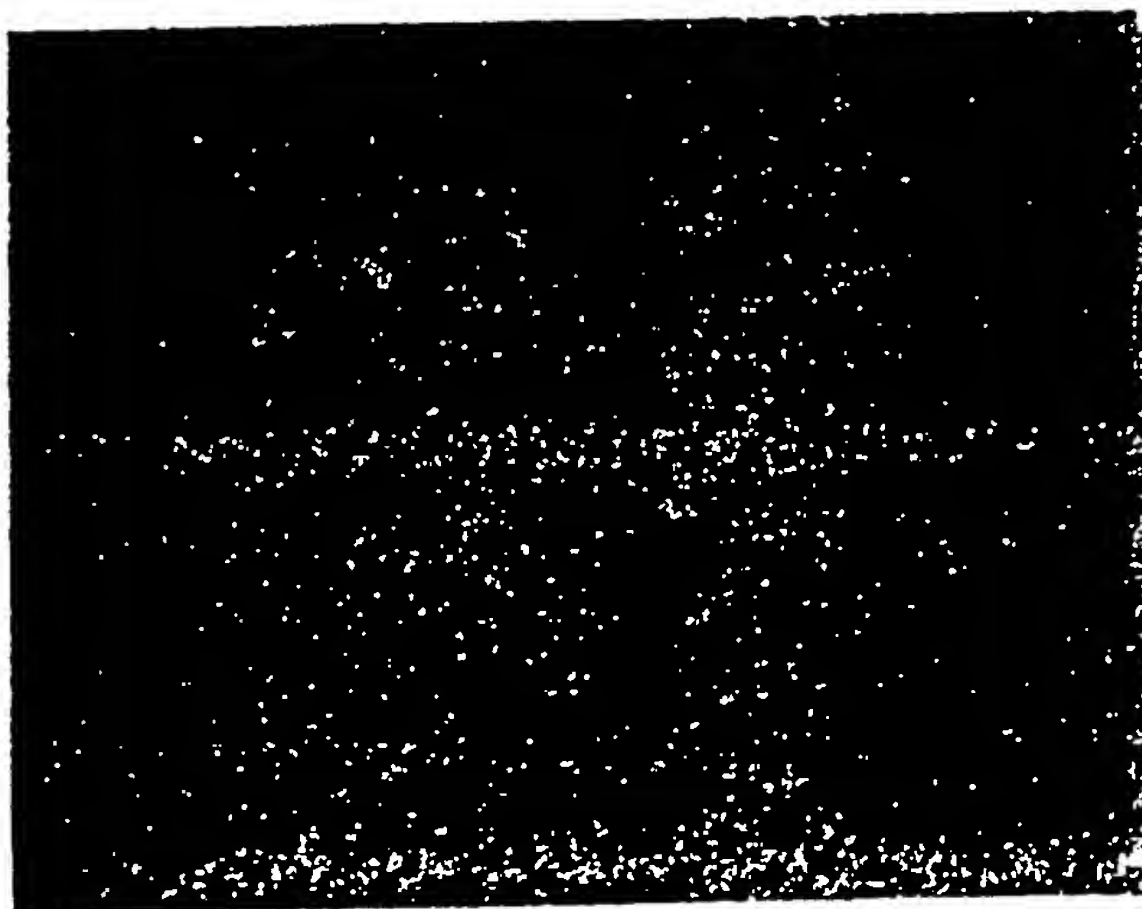
- 13 -

10. A feed pellet according to claim 9 characterized in that it contains more than 30 mg astaxanthin per kg dry matter.

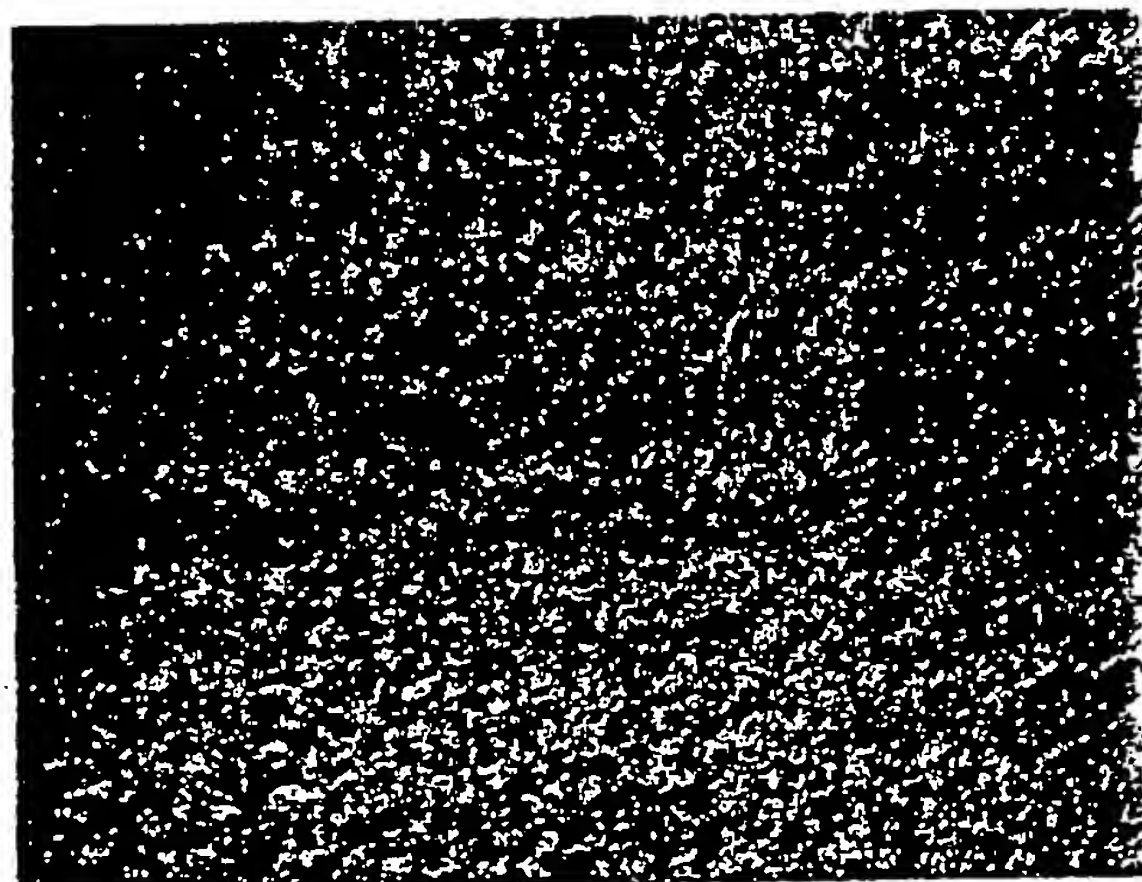


FIGURE 1/3

A



B



C



FIGURE 2/3

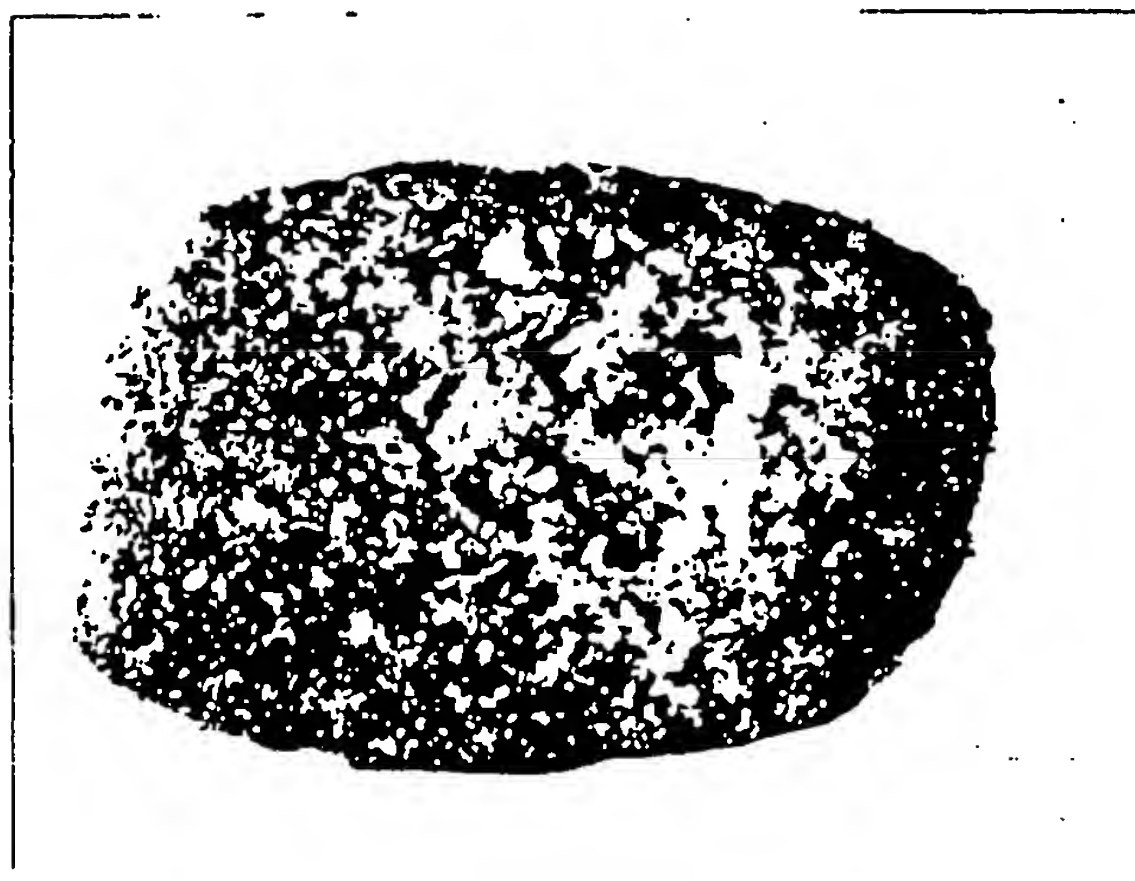
A



B



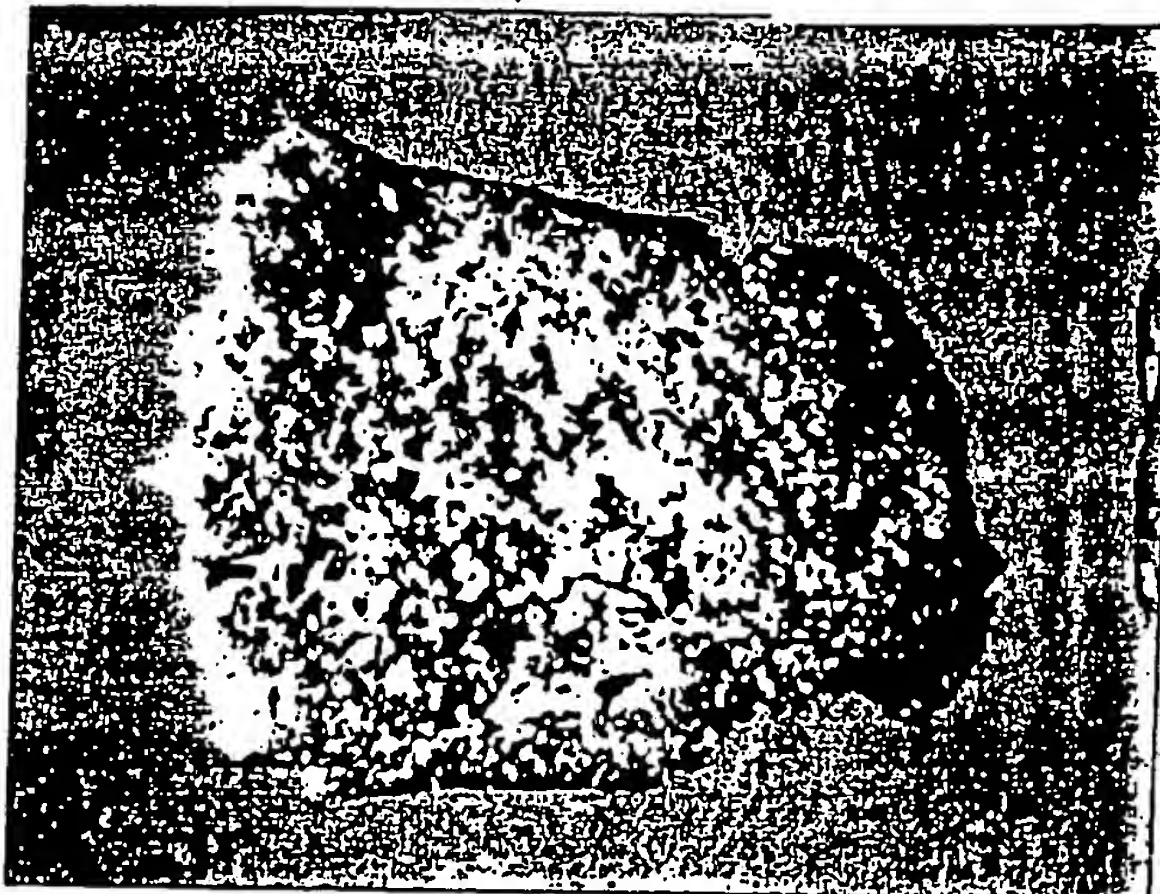
C



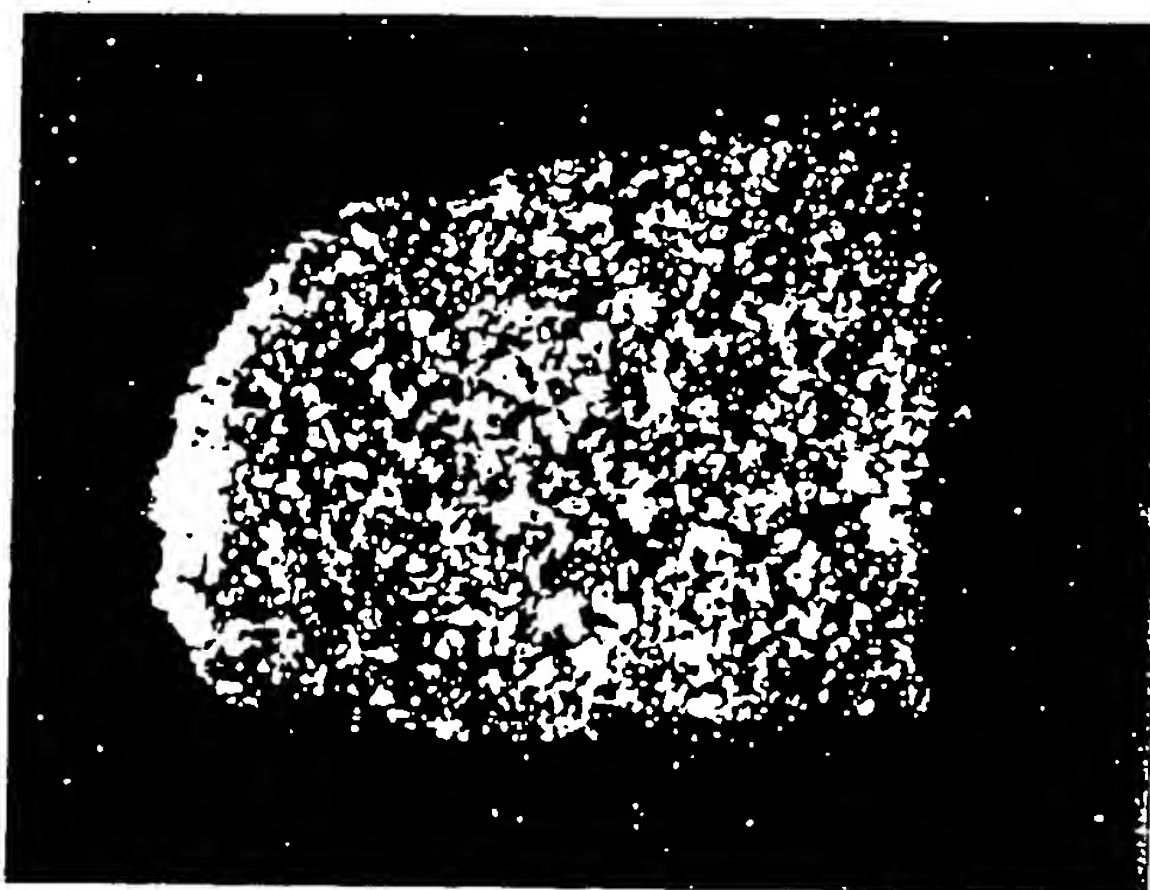
3 / 3

FIGURE 3/3

A



B



C



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/NL 93/00025

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 A23K1/16		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl. 5	A23K	
Documentation Searched other than Minimum Documentation to the extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	GB,A,1 572 761 (HENKEL) 6 August 1980 see page 2, line 22 - line 34; claims 1-8 ----	1,2
X	GB,A,985 613 (F. HOFFMANN-LA ROCHE) 10 March 1965 see claims 1,3,5; examples 7-9 ----	1,2
X	FEEDSTUFFS vol. 51, no. 3, 15 January 1979, US page 33 JAMES W. ANDREWS ET AL. 'Surface coating of fish feeds with animal fat and ascorbic acid' see the whole document ----- -/--	1
<p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
30 MARCH 1993	28.04.93	
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EUROPEAN PATENT OFFICE	DEKEIREL M.J.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category <sup>a</sup>	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	EP,A,0 231 817 (GEBRÜDER BÜHLER AG) 12 August 1987 see claims 1,2,4,5	1
Y	---	3
Y	GB,A,2 232 573 (CANADA PACKERS INC) 19 December 1990 see claims 1-14; examples 1-5	3
X	WO,A,8 808 025 (DANISCO BIOTEKNOLOGI A/S) 20 October 1988 see page 19, line 23 - line 28 see page 20, line 21 - page 21, line 2 see claim 24 see example 11	9,10
A	---	
X	FEEDSTUFFS vol. 49, no. 17, 9 May 1977, US pages 26 - 38 SAMUEL P. MEYERS 'Using crustacean meals and carotenoid-fortified diets' see page 27, column 1; table 4 see page 38, column 1, last paragraph see page 38, column 2, last paragraph - column 3, paragraph 1	9,10
A	---	
A	WO,A,8 707 116 (JESMA-MATADOR A/S) 3 December 1987 see claim	3
A	---	
A	DE,A,2 933 261 (HOLZAPFEL) 19 February 1981 see page 7, line 4 - line 35; claims 1,4-7	3
	-----	

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

NL 9300025  
SA 69917

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A-1572761	06-08-80	DE-A- 2602260 CH-A- 625943 FR-A, B 2338653 NL-A- 7614592	04-08-77 30-10-81 19-08-77 26-07-77
GB-A-985613		None	
EP-A-0231817	12-08-87	CH-A- 666386 DE-A- 3772399	29-07-88 07-11-91
GB-A-2232573	19-12-90	US-A- 4971820	20-11-90
WO-A-8808025	20-10-88	AU-B- 620034 AU-A- 1688988 EP-A- 0367765 JP-T- 2504101	13-02-92 04-11-88 16-05-90 29-11-90
WO-A-8707116	03-12-87	US-A- 4861606	29-08-89
DE-A-2933261	19-02-81	None	

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